recently, structures of the dynein motor domain have been reported at 5-6 Å resolution, which have provided invaluable information at the secondary structure level. However, a higher resolution analysis is required to reveal complete atomic structure of the motor domain.
Here we report the crystal structure of the 380-kDa motor domain of Dictyostelium cytoplasmic dynein at 2.8 Å resolution. This atomic structure shows details of functional units constituting the motor domain, such as the ATP-hydrolyzing ring composed of six AAA+ modules, the long coiled-coil microtubule-binding stalk, and the force-generating rod-like linker. Our analysis visualizes four ADP molecules bound to the first four AAA+ modules of the ring, among which three are active ATP hydrolysis site and one is a unique ADP/ATP binding site. The structure also uncovers how the linker and stalk interact with the ring unit, which should be critical for dynein's motor activity. This long sought atomic structure will open up new avenues for investigating and understanding how dynein produces force and movement.

1865-Pos Board B635
Intrinsic Disorder in the Kinesin Superfamily
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The C-terminus of conventional kinesin (Kif5B) has long been referred to in the literature as the globular tail. In this work we show that this domain is in fact intrinsically disordered. The unfolded structure of the tail domain is revealed via in silico prediction methods, and CD and NMR spectroscopies. It has been well established that a diverse collection of cargos bind exclusively to the tail domain of Kif5B, and being natively unstructured would allow the tail to sample a variety of conformations in order to accommodate these various binding-partners. Expanding the in silico prediction methods to include all members of the human kineisn superfamily, we find that significant regions of intrinsic disorder are found in the motor or stalk or tail domains of every human kinesin. These regions contain little to no sequence conservation between different molecules, vary in length and net charge, and appear to serve a variety of purposes including structural flexibility and facilitating ligand binding and post-translational modifications. Thus, intrinsic disorder appears to be a structural feature that is important for various aspects of kinesin biology.

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The Length of the Neck Linker Domain Controls Processivity Across Diverse N-Terminal Kinesin Families
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Consistent with their diverse intracellular roles, the processivity of N-terminal kinesin motors varies considerably between different families. Gating mechanisms that control kinesin processivity involve inter-head tension that must be transmitted through the neck linker domains of each head. The objective of this study is to determine the degree to which unloaded processivity is controlled by the length of the neck linker, as opposed to kinetic differences in the core motor domains. The motor and neck linker domains of Kinesin-2, 3, 5 and 7 were fused to the neck-coil and rod domains of Kinesin-1 and run lengths of GFP-tagged motors visualized by total internal fluorescence microscopy. When the neck linkers were shortened to the 14 amino acids found in Kinesin-1, all motors had similar run lengths, and when the neck linkers were extended the run lengths fell. These results suggest that inter-head tension, which is regulated by the mechanical properties of the neck linker, controls processivity in a conserved manner across diverse kinesins. Monte Carlo modeling of the kinesin hydrolysis cycle conclude that the results can be quantitatively accounted for by differences in either front head or rear head gating.

1867-Pos Board B637
Mechanism of Kinesin-13 Binding to Microtubules as Revealed by Single Molecule Fluorescence Polarization Microscopy
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The kinesin superfamily of motor proteins are involved in diverse cellular processes, including intracellular organelle transport, cell division and cytoskeletal dynamics. The widely studied conventional kinesin (or kinesin-1) translocates along the microtubule (MT) by utilizing the energy generated from ATP hydrolysis. In contrast, members of the Kinesin-13 family do not walk along the MT lattice, but use their catalytic core to rapidly target microtubule ends by the process of one-dimensional diffusion (ODD) and promote depolymerization upon reaching there. However, the reason for such a significant difference in the behavior of the structurally conserved motor domain is not clearly understood. In order to reveal the mechanistic details of the kinesin-13 action, fluorescence polarization microscopy (FPM) has been employed to probe the configuration and mobility of BSR-labeled KLP10A (*Drosophila m*. Kinesin-13) molecules interacting with microtubules in the presence of different nucleotides. Experiments are being performed with KLP10A constructs of variable lengths to identify the potent mediators of diffusive motility. Preliminary results emerging from single-molecule FPM measurements have suggested that the motor core itself can undergo ODD without the assistance from the positively-charged neck domain. In addition, data acquired at both ensemble and single-molecule levels have revealed that the orientation of the KLP10A molecule relative to the MT filament is altered by mutating the crucial residues in the tubulin-binding sites on the motor domain. The structural and functional information extracted from analyses of our experimental findings will be discussed in details.

1868-Pos Board B638
Microtubule Motor Traffic Jams
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In the cell, microtubules spread throughout the cytoplasm, creating an intracellular highway system, with plus ends pointing towards the periphery. Kinesin-1 uses energy derived from ATP hydrolysis to walk towards microtubule plus ends to deliver cargoes, such as organelles or mRNA to the cell periphery. Multiple motors often work together to transport a single cargo. Transport properties of both single kinesin motors and single cargoes carried by multiple motors have been characterized. However, these studies are typically done in dilute conditions that do not accurately represent the cellular environment, where crowding or motor exchange on cargoes can occur. To address crowding and motor exchange, we use quantum dots, known to spontaneously bind kinesin-1, as cargo in our experiments and high concentrations of kinesin-1 to mimic crowded conditions on microtubules. Our system allows cargoes to self-assemble with exchanging kinesin-1 motors as the cargo is transported. Using Total Internal Reflection Fluorescence (TIRF) Microscopy, we tracked individual cargoes over a wide range of kinesin-1 concentrations to mimic varying degrees of crowded conditions. We found that while the velocity of cargoes decreased as conditions became more crowded, the run length and total association time of cargoes increased. We observed that cargoes paused more frequently in crowded conditions. Interestingly, we also observed cargo reversal events during runs, which were more likely to occur in crowded conditions. We believe these reversals occur when multiple motors are bound to a single cargo. If the leading motor is stretched and under strain, if it detaches, the cargo will rock backwards. Using coarse-grained Brownian dynamics simulations combined with model convolution microscopy, we can recapitulate velocity reduction, increased pausing, and reversals in silico.

1869-Pos Board B639
Casein Kinase 2 Reverses Tail-Independent Inactivation of Kinesin-1
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Kinesin-1 is a plus-end microtubule-based motor, and defects in kinesin-based transport are linked to diseases including neurodegeneration. Kinesin can auto-inhibit via a direct head-tail interaction, but is believed to be active otherwise. Here we report a tail-independent inactivation of kinesin, reversible by the disease-relevant signaling protein, casein kinase 2 (CK2). The majority of initially active kinesin (native or tail-less) loses its ability to bind/interact with microtubules in vitro, and CK2 reverses this inactivation (~ 4-fold) without altering kinesin's single motor properties. This activation pathway does not require motor phosphorylation, and is independent of head-tail autoinhibition. In cultured mammalian cells, reducing CK2 expression, but not kinase activity, decreases the force required to stall lipid droplet transport, consistent with a reduction in the number of active motors. These results provide the first direct evidence of a protein kinase up-regulating kinesin-based transport, and suggest a novel pathway for regulating the activity of cargo-bound kinesin.

1870-Pos Board B640
The Relationship of Motor Attachment Geometry and Velocity Fluctuations in Kinesin-Microtubule Gliding Motility Assays
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In gliding motility assays, surface-adhered motor proteins bind to the gliding filaments in a variety of orientations. The resulting variations in the angle of